



Supplemental Figure 1. Interaction effects on A_{290} of between ascorbate , GSNO and H_2O_2 . Purified APX protein was incubated at different concentrations of SIN-1 at 37°C for 60 min, GSNO at 25°C for 30 min or GSH at 25°C for 30 min. The specific activity of the recombinant APX was 204 nmol ascorbate · min⁻¹ · mg⁻¹ protein. Data are means ± SEM of at least three replicates. *Differences from control values were significant at $P < 0.05$.

Supplemental Figure 2

Multiple sequence alignments(A) and phylogenetic tree (B) of the amino acid sequence of pea cAPX and other 15 APX sequences found in Peroxibase (<http://peroxibase.toulouse.inra.fr/>) from the three model plants *Arabidopsis thaliana*, *Medicago truncatula* and *Lotus japonicus* with different subcellular localization including cytosol, peroxisome and chloroplast (stroma and thylakoid). Six APXs are from *A. thaliana*, including two cytosolic (AtAPx01, AtAPx02), 2 peroxisomal (AtAPx03, AtAPx04), 1 stromal (AtAPx05), 1 thylakoid-bound (AtAPx06) and 1 thylakoid-lumen that is remarked as not as APX (AtAPx07); seven APXs from *M. truncatula*: 2 cytosolic (MtAPx01, MtAPx03), 2 peroxisomal (MtAPx02, MtAPx05), 2 thylakoid-bound (MtAPx07-1, MtAPx07-2) and 1 un-localized (MtAPx05); and two APXs from *L. japonicus*: peroxisomal (LjAPx02) and cytosolic (LjAPx01). The relative position of Tyr-5, Tyr-235 and Cys-32 of pea cAPX is indicated in the protein sequences by yellow color. Single asterisk indicates identical residues. Semi-colon indicates conservative substitutions. Period indicate less conservative substitutions.

(A)

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MtAPx07-1_thylakoid-bound -----MATITGGAAARMIPSATRATVSLSTSRSFSSFS
MtAPx07-2_thylakoid-bound -----MATITGGAAARMIPSATRATVSLSTSRSFSSFS
AtAPx06_thylakoid-bound -----MSVSLSAASHLLCSSTRVSLSPAVTSSSSSPVV
AtAPx05_stromal MAERVSLTLNGTLLSPPPTTTTTTMSSSLRSTTAASLLLRSSSSSRSTL
LjAPx02_peroxisomal -----
MtAPx02_peroxisomal -----
AtAPx03_peroxisomal -----
MtAPx05_peroxisomal -----
AtAPx04_peroxisomal -----
PsAPX_cytosolic -----
MtAPx01_cytosolic -----
LjAPx01_cytosolic -----
MtAPx03_cytosolic -----
AtAPx01_cytosolic -----
AtAPx02_cytosolic -----
MtAPx04_N/D -----MGVSFLSISTLPSLHPLGGSHYPKTTSTRHQPRSVSICCAKIK

MtAPx07-1_thylakoid-bound LASSRSVSSLNCLRSSPRISHIFLNQRRGEVR-----VSSGRFGTVAF
MtAPx07-2_thylakoid-bound LASSRSVSSLNCLRSSPRISHIFLNQRRGEVR-----VSSGRFGTVAF
AtAPx06_thylakoid-bound ALSSSTSPHSLGVSASSSLFPHSSFVLQKKHPIN----GTSTRMISPKCA
AtAPx05_stromal TLSASSSLSFVRSVLVSSPRLSSSSSLSQKKYRIASVNRFSNSTAATKSS
LjAPx02_peroxisomal -----MALPVVVD
MtAPx02_peroxisomal -----MAFPVVVD
AtAPx03_peroxisomal -----MAAPIVD
MtAPx05_peroxisomal -----MAKYAVD
AtAPx04_peroxisomal -----MAVNVD
PsAPX_cytosolic -----GKSYPTVS
MtAPx01_cytosolic -----MGKSYPTVS
LjAPx01_cytosolic -----MGKSYPTVS
MtAPx03_cytosolic -----M
AtAPx01_cytosolic -----MTKNYPTVS
AtAPx02_cytosolic -----MVKKSYPEVK
MtAPx04_N/D TDVSDEEEQFFHWRRLDVLKCIGLTIGLESISSGSLGTTANAADLIERR

MtAPx07-1_thylakoid-bound ASDPDQLKSAREDIKELLKTKF*CHPLLIRLGWHDAGTYNKNI EEWQRRGG
MtAPx07-2_thylakoid-bound ASDPDQLKSAREDIKELLKTKF*CHPLLIRLGWHDAGTYNKNI EEWQRRGG
AtAPx06_thylakoid-bound ASDAAQLISAKEDIKVLRLTKF*CHPILVRLGWHDAGTYNKNI EEWPLRGG
AtAPx05_stromal SSDPDQLKNAREDIKELLSTKF*CHPILVRLGWHDAGTYNKNI KEWPQRRGG
LjAPx02_peroxisomal GDYLKEVDKARRDLRALIANRNCAPLMLRLAWHDAGTYDA----RKTGTGG
MtAPx02_peroxisomal SEYLKEIEKARRDLRALIANRNCAPLMLRLAWHDAGTYDA----KTKTGG
AtAPx03_peroxisomal AEYLKEITKARRELRLSIANKNCAPIMLRLAWHDAGTYDA----QSKTGG
MtAPx05_peroxisomal AEYVKEIEEVRRDLRSFITNKK*CAPLMLRLAWHDAGTYDA----KTRTGG
AtAPx04_peroxisomal AEYLKEIEKTRDLRALISSRNCAPIMLRLAWHDAGTYDA----KKTGTGG
PsAPX_cytosolic PDYQKAIEKAKRKLGRFIAEKK*CAPLILRLAWHSAGTFDS----KTKTGG
MtAPx01_cytosolic ADYQKAVEKAKRKLGRFIAEKK*CAPLMLRLAWHSAGTFDS----KTKTGG
LjAPx01_cytosolic ADYQKAIEKARRKLGRFIADKK*CAPLMLRLAWHSAGTFDV----KTKTGG
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MtAPx03_cytosolic QTQTPALNASRKKLVELIETSRNVWLIFHWKS-----
AtAPx01_cytosolic EDYKKAWEKCRKRRLRGLIAEKNCAPIMVRLAWHSAGTFDC----QSRRTGG
AtAPx02_cytosolic EYKKAQVQRCKRKLRLGLIAEKHCAPIVLRLAWHSAGTFDV----KTKTGG
MtAPx04_N/D QRSDFQSQIKGTLTYAIKANPDLTPSILTLALNDALTYDK----ATKTGG

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MtAPx07-1_thylakoid-bound ANGSLRFEVELKHGANAGLVNALKLLQPIKDKYSGVTYADLFQLASATAV
MtAPx07-2_thylakoid-bound ANGSLRFEVELKHGANAGLVNALKLLQPIKDKYSGVTYADLFQLASATAV
AtAPx06_thylakoid-bound ANGSLRFEAEELKHAANAGLLNALKLIQPLKDKYPNISYADLFQLASATAI
AtAPx05_stromal ANGSLRFDIELKHAANAGLVNALKLLQPIKDKYSGVTYADLFQLASATAI
LjAPx02_peroxisomal PNGSIRNEEEFSGHANNGLKKAIDFCEEVKTKVPKITYADLYQLAGVVAV
MtAPx02_peroxisomal PNASIRNEEEFSGHANNGLKKAIDFCEEVKAKHPKI SYADLYQLAGVVAV
AtAPx03_peroxisomal PNGSIRNEEEHSHGANSGLKIALDLCEGVKAKHPKI SYADLYQLAGVVAV
MtAPx05_peroxisomal PNGSIRNQOELNHAANKGLKTAVELCEEVKVKHPKVSADLYQLAGVVAV
AtAPx04_peroxisomal ANGSIRFKEELNRPHNKGLEKAVAFCEEVKAKHPRVSYADLYQLAGVVAV
PsAPX_cytosolic PFGTIKHQAELAHGANGLDIAVRLLEPLKEQFPPI SYADFYQLAGVVAV
MtAPx01_cytosolic PFGTIKHQAELAHGANGLDIAVRLLEPLKEQFPPI SYADFYQLAGVVAV
LjAPx01_cytosolic PFGTMKHAELAHGANGLDIAVRLLEPLKEQFPPI SYADFYQLAGVVAV
MtAPx03_cytosolic -----KVILEPLKEQFLIISYVDFYQLSGVVAV
AtAPx01_cytosolic PFGTMRFDAEQAHGANSGLIHALRLLDPIREQFPPI SFADFHQLAGVVAV
AtAPx02_cytosolic PFGTIRHPQELAHGANGLDIAVRLLEPLKEQFPPI SYADFYQLAGVVAV
MtAPx04_N/D PNGSIRFSSEISRPNKGLSALNFIEEAKKEIDSYSGKGPISYADLIQY

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MtAPx07-1_thylakoid-bound EEAGGPKIPMKYGRVDVTGPEQCPEEGRLPDAGPPSPADHLRQVIFY-RMG
MtAPx07-2_thylakoid-bound EEAGGPKIPMKYGRVDVTGPEQCPEEGRLPDAGPPSPADHLRQVIFY-RMG
AtAPx06_thylakoid-bound EEAGGPDIPMKYGRVDVVAPEQCPEEGRLPDAGPPSPADHLRDVIFY-RMG
AtAPx05_stromal EEAGGPKIPMKYGRVDASGPEDCPEEGRLPDAGPPSPATHLREVFIFY-RMG
LjAPx02_peroxisomal EITGGPTINFPVGRKDS---KISTNEGRLPDA--KQGVPHLRDIFY-RMG
MtAPx02_peroxisomal EVTGGPTVNFVPGRRDS---KVCTRDGRLPDA--KQGVSHLRDIFY-RMG
AtAPx03_peroxisomal EVTGGPDIVFVPGRRDS---NVCPEGRRLPDA--KQGFQHLRDVIFY-RMG
MtAPx05_peroxisomal EVTGGPTIQFVPGRRDS---LESPQEGRLPDA--KQGASHLREIFY-RMG
AtAPx04_peroxisomal EVTGGPAIPFTVGRKDS---ADSADDGELPNP--NEGASHLRTLFS-RMG
PsAPX_cytosolic EITGGPEVFPFHGREDK---PEPPPEGRRLPDA--TKGSDHLRDVFGKAMG
MtAPx01_cytosolic EVTGGPEVFPFHGREDK---PEPPPEGRRLPDA--TKGSDHLRDVFGKAMG
LjAPx01_cytosolic EVTGGPDVFPFHGREDK---PQPPPEGRRLPDA--TKGSDHLRDVFKVTMG
MtAPx03_cytosolic EITGGPEVFPFHGGEDK---PEPPLEGRRLPDA--TEGSNHLRDVFGKSMG
AtAPx01_cytosolic EVTGGPDIPFHPGREDK---PQPPPEGRRLPDA--TKGCDHLRDVFAKQMG
AtAPx02_cytosolic EITGGPEIPFHPGRLDK---VEPPPEGRRLPDA--TKGVDHLRDVFG-RMG
MtAPx04_N/D AAQSATKAQFLASAIRK---CGGNEEKGNLLYT--AYGSNGQWGLFDRQFG

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MtAPx07-1_thylakoid-bound LNDKEIVALSGAHTLGRSRPDRSGWGKPKETKYTKDGPAGGGQSWTAQWL
MtAPx07-2_thylakoid-bound LNDKEIVALSGAHTLGRSRPDRSGWGKPKETKYTKDGPAGGGQSWTAQWL
AtAPx06_thylakoid-bound LDDKEIVALSGAHTLGRARPDRSGWGKPKETKYTKTGPGEAGGGQSWTVKWL
AtAPx05_stromal LDDKDIVALSGAHTLGRSRPDRSGWGKPKETKYTKEGPGAPGGQSWTPEWL
LjAPx02_peroxisomal LTDKDIVALSGAHTLGRAHPERSGFDFG-----WTEEDPL
MtAPx02_peroxisomal LTDKDIVALSGAHTLGRAHPERSGFDFG-----WTEEDPL
AtAPx03_peroxisomal LSDKDIVALSGGHTLGRAHPERSGFDFG-----WTQEPL
MtAPx05_peroxisomal LTDKDIVALSGGHTLGKAHKDRSNFEGQ-----WTRDPL
AtAPx04_peroxisomal LLDRDIVALSGGHTLGRAHKERSDFEGP-----WTQDPL
PsAPX_cytosolic LSDQDIVALSGGHTIGAAHKERSGFEGP-----WTSNPL
MtAPx01_cytosolic LSDQDIVALSGGHTIGAAHKERSGFEGP-----WTSNPL
LjAPx01_cytosolic LNDVDIVALSGGHTLGAHKERSGFEGP-----WTTNPH
MtAPx03_cytosolic LSDQDIVALSGXHTIGAAHKERSGFEGP-----WTSNPL
AtAPx01_cytosolic LSDKDIVALSGAHTLGRCHKDRSGFEGA-----WTSNPL
AtAPx02_cytosolic LNDKDIVALSGGHTLGRCHKERSGFEGA-----WTPNPL
MtAPx04_N/D RTDTDEADPEGRIPLWEKASVQEMKDKFS-----AVGFG

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MtAPx07-1_thylakoid-bound KFDNSYFKDIKEKKDEDLLVLPDAAALFDDPSFKVYAEKYAVDQEAFFKD
MtAPx07-2_thylakoid-bound KFDNSYFKDIKEKKDEDLLVLPDAAALFDDPSFKVYAEKYAVDQEAFFKD
AtAPx06_thylakoid-bound KFDNSYFKDIKEKRDDLLVLPDAAALFDDPSFKVYAEKYAEDVAAFFKD
AtAPx05_stromal KFDNSYFKEIKEKRDEDLLVLPDAAALFDDPSFKVYAEKYAADQDAFFKD
LjAPx02_peroxisomal KFDNSYFVELLKEESAGLLKLPDALKALLEDPAFRRYVELYAKDEEAFFRD
MtAPx02_peroxisomal KFDNSYFQILLEEDSAAALLKLPDTRALLDDPEFRRYVELYAKDEDAFFRD
AtAPx03_peroxisomal KFDNSYFVELLKGESGLLKLPDCKLLEDPEFRRLVELYAKDEDAFFRD
MtAPx05_peroxisomal KFDNSYFVELLMKLESNDLLKLPDALKALLEDPAFRRYVELYAKDENAFFRD
AtAPx04_peroxisomal KFDNSYFVELLKGEPGLLQKLPDALKALLEDPKFHPFVKLYAKDEDMFFKA
PsAPX_cytosolic IFDNSYFTELLTGKEDGLLQLPDALKALLEDVFRPLVEKYAAEDVFFAD
MtAPx01_cytosolic IFDNSYFTELLGGEKEGLLQLPDALKALLEDVFRPLVEKYAAEDDAFFAD

LjAPx01_cytosolic IFDNTYFTELLTGEKEGLLQLPTDKALLSDPVFRPLVDKYAADEDAFFAD
 MtAPx03_cytosolic IFDNSYFTKLLGGEKEGLLQLPSDKALLSDLVFRLLVEKYVADEDAFFAD
 AtAPx01_cytosolic IFDNSYFKELLSGEKEGLLQLVSDKALLDDPVFRPLVEKYAADEDAFFAD
 AtAPx02_cytosolic IFDNSYFKEILSGEKEGLLQLPTDKALLDDPLFLPFVEKYAADEDAFFED
 MtAPx04_N/D PRQLAVLSAFIGPDQDATETLLASDPDVAPWVQKYQRSRETVSRTDYEVD
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MtAPx07-1_thylakoid-bound YAEAHAKLSNLGAKFEPAGVVDGSPNVVGEKFVAAKYSSGK-----
 MtAPx07-2_thylakoid-bound YAEAHAKLSNLGAKFEPAGVVDGSPNVVGEKFVAAKYSSGKELSDAM
 AtAPx06_thylakoid-bound YAEAHAKLSNLGAKFDPPGIVIEEN----VPEKFVAAKYSTGKKELSDSM
 AtAPx05_stromal YAVAHAKLSNLGAEFNPPEGIVI-----
 LjAPx02_peroxisomal YAESHKKLSELGFVFP-SSKASSPKDGTILLQSAVGVVVVTGAVVILSYLYE
 MtAPx02_peroxisomal YAESHKKLSELGFAP-SSKATAPVDATVLAQSAFGVAAAAVVILGYLYE
 AtAPx03_peroxisomal YAESHKKLSELGFNPSSAGKAVADSTILAQSAFGVAVAAA VVAFGYFYE
 MtAPx05_peroxisomal YATSHKKLSELGFNP-----SCSYRSQLAKAALGMVIASTVVVLGYLLE
 AtAPx04_peroxisomal YAI SHKKLSELGFNP-----PRRIPSAVTQQTGLGIAVAAA VVIF TICYE
 PsAPX_cytosolic YAEAHLKSELGFAEA-----
 MtAPx01_cytosolic YAEAHQKSELGFAEA-----
 LjAPx01_cytosolic YAESHKRLSELGFADA-----
 MtAPx03_cytosolic YVEARQKLFELGFAEA-----
 AtAPx01_cytosolic YAEAHMKLSELGFADA-----
 AtAPx02_cytosolic YTEAHLKSELGFADKE-----
 MtAPx04_N/D LITTFTKLSTLGQKINYEAYTYPRKKIDITKLL-----
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MtAPx07-1_thylakoid-bound -----
 MtAPx07-2_thylakoid-bound RKKIRAEYEAVGGSPDKALKSNYFLNIIIVIAALAILTYLFGN-----
 AtAPx06_thylakoid-bound KKKIRAEYEAIIGGSPDKPLPTNYFLNIIAIGVLVLLSTLFGGNNNSDFS
 AtAPx05_stromal -----
 LjAPx02_peroxisomal IRKRAK-----
 MtAPx02_peroxisomal THKRGK-----
 AtAPx03_peroxisomal IRKRMK-----
 MtAPx05_peroxisomal LNKKIN-----
 AtAPx04_peroxisomal ASRRGK-----
 PsAPX_cytosolic -----
 MtAPx01_cytosolic -----
 LjAPx01_cytosolic -----
 MtAPx03_cytosolic -----
 AtAPx01_cytosolic -----
 AtAPx02_cytosolic -----
 MtAPx04_N/D -----

MtAPx07-1_thylakoid-bound --
 MtAPx07-2_thylakoid-bound --
 AtAPx06_thylakoid-bound GF
 AtAPx05_stromal --
 LjAPx02_peroxisomal --
 MtAPx02_peroxisomal --
 AtAPx03_peroxisomal --
 MtAPx05_peroxisomal --
 AtAPx04_peroxisomal --
 PsAPX_cytosolic --
 MtAPx01_cytosolic --
 LjAPx01_cytosolic --
 MtAPx03_cytosolic --
 AtAPx01_cytosolic --
 AtAPx02_cytosolic --
 MtAPx04_N/D --

(B)

